# TRANSLATOR'S DECLARATION

I, Janet Hope, BSc(Hons.), MIL., MITI., translator to Messrs. Taylor and Meyer of 20 Kingsmead Road, London, SW2 3JD, Great Britain, verify that I know well both the German and the English language, that I have prepared the attached English translation of 33 pages of a German Patent application in the German language with the title:

Neue für das deaD-Gen kodierende Nukleotidsequenzen

identified by the code number 000557 BT at the upper left of each page and that the attached English translation of this document is a true and correct translation of the document attached thereto to the best of my knowledge and belief.

I further declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that wilful false statements and the like are punishable by fine or imprisonment, or both, under 18 USC 1001, and that such false statements may jeopardize the validity of this document.

Signed: 

Dated: 28 January 2004

# FEDERAL REPUBLIC OF GERMANY

## Certificate of Priority for Filing of a Patent Application

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**The attached papers are a true and accurate reproduction of the original documents for this patent application.**

Munich, 2nd August 2001

**On behalf of the President of the German Patent and Trade Mark Office**

*(signature)*

Jerofsky

# New nucleotide sequences which code for the deaD gene

The invention provides nucleotide sequences from coryneform bacteria which code for the deaD gene and a process for the fermentative preparation of amino acids using bacteria in which the deaD gene is attenuated.

Prior art

L-Amino acids, in particular L-lysine, are used in human medicine and in the pharmaceuticals industry, in the foodstuffs industry and very particularly in animal nutrition.

It is known that amino acids are prepared by fermentation from strains of coryneform bacteria, in particular Corynebacterium glutamicum. Because of their great importance, work is constantly being undertaken to improve the preparation processes. Improvements to the process can relate to fermentation measures, such as, for example, stirring and supply of oxygen, or the composition of the nutrient media, such as, for example, the sugar concentration during the fermentation, or the working up to the product form by, for example, ion exchange chromatography, or the intrinsic output properties of the microorganism itself.

Methods of mutagenesis, selection and mutant selection are used to improve the output properties of these microorganisms. Strains which are resistant to antimetabolites or are auxotrophic for metabolites of regulatory importance and which produce amino acids are obtained in this manner.

Methods of the recombinant DNA technique have also been employed for some years for improving the strain of Corynebacterium strains which produce L-amino acid, by

amplifying individual amino acid biosynthesis genes and investigating the effect on the amino acid production.

Object of the invention

The inventors had the object of providing new measures for improved fermentative preparation of amino acids.

Description of the invention

Where L-amino acids or amino acids are mentioned in the following, this means one or more amino acids, including their salts, chosen from the group consisting of L-asparagine, L-threonine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophan and L-arginine. L-Lysine is particularly preferred.

When L-lysine or lysine are mentioned in the following, not only the bases but also the salts, such as e.g. lysine monohydrochloride or lysine sulfate, are meant by this.

The invention provides an isolated polynucleotide from coryneform bacteria, comprising a polynucleotide sequence which codes for the deaD gene, chosen from the group consisting of

a) polynucleotide which is identical to the extent of at least 70% to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2,

b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70% to the amino acid sequence of SEQ ID No. 2,

c) polynucleotide which is complementary to the polynucleotides of a) or b), and

d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b) or c),

the polypeptide preferably having the activity of DNA/RNA helicase.

The invention also provides the abovementioned polynucleotide, this preferably being a DNA which is capable of replication, comprising:

(i) the nucleotide sequence, shown in SEQ ID No.1, or

(ii) at least one sequence which corresponds to sequence (i) within the range of the degeneration of the genetic code, or

(iii) at least one sequence which hybridizes with the sequences complementary to sequences (i) or (ii), and optionally

(iv) sense mutations of neutral function in (i).

The invention also provides:

a polynucleotide, in particular DNA, which is capable of replication and comprises the nucleotide sequence as shown in SEQ ID No.1;

a polynucleotide which codes for a polypeptide which comprises the amino acid sequence as shown in SEQ ID No. 2;

a vector containing parts of the polynucleotide according to the invention, but at least 15 successive nucleotides of the sequence claimed,

and coryneform bacteria in which the deaD gene is attenuated, in particular by an insertion or deletion.

The invention also provides polynucleotides, which substantially comprise a polynucleotide sequence, which are obtainable by screening by means of hybridization of a corresponding gene library of a coryneform bacterium, which comprises the complete gene or parts thereof, with a probe which comprises the sequence of the polynucleotide according to the invention according to SEQ ID No.1 or a fragment thereof, and isolation of the polynucleotide sequence mentioned.

Polynucleotides which comprise the sequences according to the invention are suitable as hybridization probes for RNA, cDNA and DNA, in order to isolate, in the full length, nucleic acids or polynucleotides or genes which code for DNA/RNA helicase or to isolate those nucleic acids or polynucleotides or genes which have a high similarity with the sequence of the deaD gene.

Polynucleotides which comprise the sequences according to the invention are furthermore suitable as primers with the aid of which DNA of genes which code for DNA/RNA helicase can be prepared by the polymerase chain reaction (PCR).

Such oligonucleotides which serve as probes or primers comprise at least 30, preferably at least 20, very particularly preferably at least 15 successive nucleotides. Oligonucleotides with a length of at least 40 or 50 nucleotides are also suitable.

"Isolated" means separated out of its natural environment.

"Polynucleotide" in general relates to polyribonucleotides and polydeoxyribonucleotides, it being possible for these to be non-modified RNA or DNA or modified RNA or DNA.

The polynucleotides according to the invention include a polynucleotide according to SEQ ID No. 1 or a fragment prepared therefrom and also those which are at least 70%, preferably at least 80% and in particular at least 90% to

95% identical to the polynucleotide according to SEQ ID No. 1 or a fragment prepared therefrom.

"Polypeptides" are understood as meaning peptides or proteins which comprise two or more amino acids bonded via peptide bonds.

The polypeptides according to the invention include a polypeptide according to SEQ ID No. 2, in particular those with the biological activity of DNA/RNA helicase and also those which are at least 70%, preferably at least 80% and in particular at least 90% to 95% identical to the polypeptide according to SEQ ID No. 2 and have the activity mentioned.

The invention furthermore relates to a process for the fermentative preparation of amino acids chosen from the group consisting of L-asparagine, L-threonine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophan and L-arginine using coryneform bacteria which in particular already produce amino acids and in which the nucleotide sequences which code for the deaD gene are attenuated, in particular eliminated or expressed at a low level.

The term "attenuation" in this connection describes the reduction or elimination of the intracellular activity of one or more enzymes (proteins) in a microorganism which are coded by the corresponding DNA, for example by using a weak promoter or using a gene or allele which codes for a corresponding enzyme with a low activity or inactivates the corresponding gene or enzyme (protein), and optionally combining these measures.

The microorganisms provided by the present invention can prepare amino acids from glucose, sucrose, lactose, fructose, maltose, molasses, starch, cellulose or from

glycerol and ethanol. They can be representatives of coryneform bacteria, in particular of the genus Corynebacterium. Of the genus Corynebacterium, there may be mentioned in particular the species Corynebacterium glutamicum, which is known among experts for its ability to produce L-amino acids.

Suitable strains of the genus Corynebacterium, in particular of the species Corynebacterium glutamicum (C. glutamicum), are in particular the known wild-type strains

Corynebacterium glutamicum ATCC13032
Corynebacterium acetoglutamicum ATCC15806
Corynebacterium acetoacidophilum ATCC13870
Corynebacterium melassecola ATCC17965
Corynebacterium thermoaminogenes FERM BP-1539
Brevibacterium flavum ATCC14067
Brevibacterium lactofermentum ATCC13869 and
Brevibacterium divaricatum ATCC14020

and L-amino acid-producing mutants or strains prepared therefrom.

The new deaD gene from C. glutamicum which codes for the enzyme DNA/RNA helicase has been isolated.

To isolate the deaD gene or also other genes of C. glutamicum, a gene library of this microorganism is first set up in Escherichia coli (E. coli). The setting up of gene libraries is described in generally known textbooks and handbooks. The textbook by Winnacker: Gene und Klone, Eine Einführung in die Gentechnologie (Verlag Chemie, Weinheim, Germany, 1990), or the handbook by Sambrook et al.: Molecular Cloning, A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1989) may be mentioned as an example. A well-known gene library is that of the E. coli K-12 strain W3110 set up in λ vectors by Kohara et al. (Cell 50, 495 -508 (1987)). Bathe et al. (Molecular and

General Genetics, 252:255-265, 1996) describe a gene library of C. glutamicum ATCC13032, which was set up with the aid of the cosmid vector SuperCos I (Wahl et al., 1987, Proceedings of the National Academy of Sciences USA, 84:2160-2164) in the E. coli K-12 strain NM554 (Raleigh et al., 1988, Nucleic Acids Research 16:1563-1575).

Börmann et al. (Molecular Microbiology 6(3), 317-326)) (1992)) in turn describe a gene library of C. glutamicum ATCC13032 using the cosmid pHC79 (Hohn and Collins, 1980, Gene 11, 291-298).

To prepare a gene library of C. glutamicum in E. coli it is also possible to use plasmids such as pBR322 (Bolivar, 1979, Life Sciences, 25, 807-818) or pUC9 (Vieira et al., 1982, Gene, 19:259-268). Suitable hosts are, in particular, those E. coli strains which are restriction- and recombination-defective, such as, for example, the strain DH5αmcr, which has been described by Grant et al. (Proceedings of the National Academy of Sciences USA, 87 (1990) 4645-4649). The long DNA fragments cloned with the aid of cosmids or other λ vectors can then in turn be subcloned and subsequently sequenced in the usual vectors which are suitable for DNA sequencing, such as is described e. g. by Sanger et al. (Proceedings of the National Academy of Sciences of the United States of America, 74:5463-5467, 1977).

The resulting DNA sequences can then be investigated with known algorithms or sequence analysis programs, such as e.g. that of Staden (Nucleic Acids Research 14, 217-232(1986)), that of Marck (Nucleic Acids Research 16, 1829-1836 (1988)) or the GCG program of Butler (Methods of Biochemical Analysis 39, 74-97 (1998)).

The new DNA sequence of C. glutamicum which codes for the deaD gene and which, as SEQ ID No. 1, is a constituent of the present invention has been found. The amino acid

sequence of the corresponding protein has furthermore been derived from the present DNA sequence by the methods described above. The resulting amino acid sequence of the deaD gene product is shown in SEQ ID No. 2.

Coding DNA sequences which result from SEQ ID No. 1 by the degeneracy of the genetic code are also a constituent of the invention. In the same way, DNA sequences which hybridize with SEQ ID No. 1 or parts of SEQ ID No. 1 are a constituent of the invention. Conservative amino acid exchanges, such as e.g. exchange of glycine for alanine or of aspartic acid for glutamic acid in proteins, are furthermore known among experts as "sense mutations" which do not lead to a fundamental change in the activity of the protein, i.e. are of neutral function. It is furthermore known that changes on the N and/or C terminus of a protein cannot substantially impair or can even stabilize the function thereof. Information in this context can be found by the expert, inter alia, in Ben-Bassat et al. (Journal of Bacteriology 169:751-757 (1987)), in O'Regan et al. (Gene 77:237-251 (1989)), in Sahin-Toth et al. (Protein Sciences 3:240-247 (1994)), in Hochuli et al. (Bio/Technology 6:1321-1325 (1988)) and in known textbooks of genetics and molecular biology. Amino acid sequences which result in a corresponding manner from SEQ ID No. 2 are also a constituent of the invention.

In the same way, DNA sequences which hybridize with SEQ ID No. 1 or parts of SEQ ID No. 1 are a constituent of the invention. Finally, DNA sequences which are prepared by the polymerase chain reaction (PCR) using primers which result from SEQ ID No. 1 are a constituent of the invention. Such oligonucleotides typically have a length of at least 15 nucleotides.

Instructions for identifying DNA sequences by means of hybridization can be found by the expert, inter alia, in the handbook "The DIG System Users Guide for Filter

Hybridization" from Boehringer Mannheim GmbH (Mannheim, Germany, 1993) and in Liebl et al. (International Journal of Systematic Bacteriology 41: 255-260 (1991)). The hybridization takes place under stringent conditions, that is to say only hybrids in which the probe and target sequence, i. e. the polynucleotides treated with the probe, are at least 70% identical are formed. It is known that the stringency of the hybridization, including the washing steps, is influenced or determined by varying the buffer composition, the temperature and the salt concentration. The hybridization reaction is preferably carried out under a relatively low stringency compared with the washing steps (Hybaid Hybridisation Guide, Hybaid Limited, Teddington, UK, 1996).

A 5x SSC buffer at a temperature of approx. 50°C - 68°C, for example, can be employed for the hybridization reaction. Probes can also hybridize here with polynucleotides which are less than 70% identical to the sequence of the probe. Such hybrids are less stable and are removed by washing under stringent conditions. This can be achieved, for example, by lowering the salt concentration to 2x SSC and optionally subsequently 0.5x SSC (The DIG System User's Guide for Filter Hybridisation, Boehringer Mannheim, Mannheim, Germany, 1995) a temperature of approx. 50°C - 68°C being established. It is optionally possible to lower the salt concentration to 0.1x SSC. Polynucleotide fragments which are, for example, at least 70% or at least 80% or at least 90% to 95% identical to the sequence of the probe employed can be isolated by increasing the hybridization temperature stepwise from 50°C to 68°C in steps of approx. 1 - 2°C. Further instructions on hybridization are obtainable on the market in the form of so-called kits (e.g. DIG Easy Hyb from Roche Diagnostics GmbH, Mannheim, Germany, Catalogue No. 1603558).

Instructions for amplification of DNA sequences with the aid of the polymerase chain reaction (PCR) can be found by the expert, inter alia, in the handbook by Gait: Oligonucleotide Synthesis: A Practical Approach (IRL Press, Oxford, UK, 1984) and in Newton and Graham: PCR (Spektrum Akademischer Verlag, Heidelberg, Germany, 1994).

It has been found that coryneform bacteria produce amino acids in an improved manner after attenuation of the deaD gene.

To achieve an attenuation, either the expression of the deaD gene or the catalytic properties of the enzyme protein can be reduced or eliminated. The two measures can optionally be combined.

The reduction in gene expression can take place by suitable culturing or by genetic modification (mutation) of the signal structures of gene expression. Signal structures of gene expression are, for example, repressor genes, activator genes, operators, promoters, attenuators, ribosome binding sites, the start codon and terminators. The expert can find information on this e.g. in patent application WO 96/15246, in Boyd and Murphy (Journal of Bacteriology 170: 5949 (1988)), in Voskuil and Chambliss (Nucleic Acids Research 26: 3548 (1998), in Jensen and Hammer (Biotechnology and Bioengineering 58: 191 (1998)), in Pátek et al. (Microbiology 142: 1297 (1996)), Vasicova et al. (Journal of Bacteriology 181: 6188 (1999)) and in known textbooks of genetics and molecular biology, such as e.g. the textbook by Knippers ("Molekulare Genetik", 6th edition, Georg Thieme Verlag, Stuttgart, Germany, 1995) or that by Winnacker ("Gene und Klone", VCH Verlagsgesellschaft, Weinheim, Germany, 1990).

Mutations which lead to a change or reduction in the catalytic properties of enzyme proteins are known from the prior art; examples which may be mentioned are the works by

Qiu and Goodman (Journal of Biological Chemistry 272: 8611-8617 (1997)), Sugimoto et al. (Bioscience Biotechnology and Biochemistry 61: 1760-1762 (1997)) and Möckel ("Die Threonindehydratase aus Corynebacterium glutamicum: Aufhebung der allosterischen Regulation und Struktur des Enzyms", Reports from the Jülich Research Centre, Jül-2906, ISSN09442952, Jülich, Germany, 1994). Summarizing descriptions can be found in known textbooks of genetics and molecular biology, such as e.g. that by Hagemann ("Allgemeine Genetik", Gustav Fischer Verlag, Stuttgart, 1986).

Possible mutations are transitions, transversions, insertions and deletions. Depending on the effect of the amino acid exchange on the enzyme activity, "missense mutations" or "nonsense mutations" are referred to. Insertions or deletions of at least one base pair (bp) in a gene lead to frame shift mutations, as a consequence of which incorrect amino acids are incorporated or translation is interrupted prematurely. Deletions of several codons typically lead to a complete loss of the enzyme activity. Instructions on generation of such mutations are prior art and can be found in known textbooks of genetics and molecular biology, such as e.g. the textbook by Knippers ("Molekulare Genetik", 6th edition, Georg Thieme Verlag, Stuttgart, Germany, 1995), that by Winnacker ("Gene und Klone", VCH Verlagsgesellschaft, Weinheim, Germany, 1990) or that by Hagemann ("Allgemeine Genetik", Gustav Fischer Verlag, Stuttgart, 1986).

A common method of mutating genes of C. glutamicum is the method of "gene disruption" and "gene replacement" described by Schwarzer and Pühler (Bio/Technology 9, 84-87 (1991)).

In the method of gene disruption a central part of the coding region of the gene of interest is cloned in a plasmid vector which can replicate in a host (typically E.

coli), but not in C. glutamicum. Possible vectors are, for example, pSUP301 (Simon et al., Bio/Technology 1, 784-791 (1983)), pK18mob or pK19mob (Schäfer et al., Gene 145, 69-73 (1994)), pK18mobsacB or pK19mobsacB (Jäger et al., Journal of Bacteriology 174: 5462-65 (1992)), pGEM-T (Promega corporation, Madison, WI, USA), pCR2.1-TOPO (Shuman (1994). Journal of Biological Chemistry 269:32678-84; US Patent 5,487,993), pCR®Blunt (Invitrogen, Groningen, Holland; Bernard et al., Journal of Molecular Biology, 234: 534-541 (1993)) or pEM1 (Schrumpf et al, 1991, Journal of Bacteriology 173:4510-4516). The plasmid vector which contains the central part of the coding region of the gene is then transferred into the desired strain of C. glutamicum by conjugation or transformation. The method of conjugation is described, for example, by Schäfer et al. (Applied and Environmental Microbiology 60, 756-759 (1994)). Methods for transformation are described, for example, by Thierbach et al. (Applied Microbiology and Biotechnology 29, 356-362 (1988)), Dunican and Shivnan (Bio/Technology 7, 1067-1070 (1989)) and Tauch et al. (FEMS Microbiological Letters 123, 343-347 (1994)). After homologous recombination by means of a "cross-over" event, the coding region of the gene in question is interrupted by the vector sequence and two incomplete alleles are obtained, one lacking the 3' end and one lacking the 5' end. This method has been used, for example, by Fitzpatrick et al. (Applied Microbiology and Biotechnology 42, 575-580 (1994)) to eliminate the recA gene of C. glutamicum.

In the method of "gene replacement", a mutation, such as e.g. a deletion, insertion or base exchange, is established in vitro in the gene of interest. The allele prepared is in turn cloned in a vector which is not replicative for C. glutamicum and this is then transferred into the desired host of C. glutamicum by transformation or conjugation. After homologous recombination by means of a first "cross-over" event which effects integration and a suitable second

"cross-over" event which effects excision in the target gene or in the target sequence, the incorporation of the mutation or of the allele is achieved. This method was used, for example, by Peters-Wendisch et al. (Microbiology 144, 915 - 927 (1998)) to eliminate the pyc gene of C. glutamicum by a deletion.

A deletion, insertion or a base exchange can be incorporated into the deaD gene in this manner.

In addition, it may be advantageous for the production of L-amino acids to enhance, in particular over-express, one or more enzymes of the particular biosynthesis pathway, of glycolysis, of anaplerosis, of the citric acid cycle, of the pentose phosphate cycle, of amino acid export and optionally regulatory proteins, in addition to the attenuation of the deaD gene.

Thus, for the preparation of L-amino acids, in addition to the attenuation of the deaD gene at the same time one or more of the genes chosen from the group consisting of

- the dapA gene which codes for dihydrodipicolinate synthase (EP-B 0 197 335),
- the gap gene which codes for glyceraldehyde 3-phosphate dehydrogenase (Eikmanns (1992), Journal of Bacteriology 174:6076-6086),
- the tpi gene which codes for triose phosphate isomerase (Eikmanns (1992), Journal of Bacteriology 174:6076-6086),
- the pgk gene which codes for 3-phosphoglycerate kinase (Eikmanns (1992), Journal of Bacteriology 174:6076-6086),
- the zwf gene which codes for glucose 6-phosphate dehydrogenase (JP-A-09224661),
- the pyc gene which codes for pyruvate carboxylase (DE-A-198 31 609),

- the mqo gene which codes for malate-quinone oxidoreductase (Molenaar et al., European Journal of Biochemistry 254, 395-403 (1998)),
- the lysC gene which codes for a feed-back resistant aspartate kinase (Accession No.P26512),
- the lysE gene which codes for lysine export (DE-A-195 48 222),
- the hom gene which codes for homoserine dehydrogenase (EP-A 0131171),
- the ilvA gene which codes for threonine dehydratase (Möckel et al., Journal of Bacteriology (1992) 8065-8072)) or the ilvA(Fbr) allele which codes for a "feed back resistant" threonine dehydratase (Möckel et al., (1994) Molecular Microbiology 13: 833-842),
- the ilvBN gene which codes for acetohydroxy-acid synthase (EP-B 0356739),
- the ilvD gene which codes for dihydroxy-acid dehydratase (Sahm and Eggeling (1999) Applied and Environmental Microbiology 65: 1973-1979),
- the zwa1 gene which codes for the Zwa1 protein (DE: 19959328.0, DSM 13115)

can be enhanced, in particular over-expressed.

It may furthermore be advantageous for the production of amino acids, in addition to the attenuation of the deaD gene, at the same time for one or more of the genes chosen from the group consisting of

- the pck gene which codes for phosphoenol pyruvate carboxykinase (DE 199 50 409.1, DSM 13047),

- the pgi gene which codes for glucose 6-phosphate isomerase (US 09/396,478, DSM 12969),
- the poxB gene which codes for pyruvate oxidase (DE:1995 1975.7, DSM 13114),
- the zwa2 gene which codes for the Zwa2 protein (DE: 19959327.2, DSM 13113)

to be attenuated, in particular for the expression thereof to be reduced.

In addition to the attenuation of the deaD gene it may furthermore be advantageous for the production of amino acids to eliminate undesirable side reactions (Nakayama: "Breeding of Amino Acid Producing Microorganisms", in: Overproduction of Microbial Products, Krumphanzl, Sikyta, Vanek (eds.), Academic Press, London, UK, 1982).

The invention also provides the microorganisms prepared according to the invention, and these can be cultured continuously or discontinuously in the batch process (batch culture) or in the fed batch (feed process) or repeated fed batch process (repetitive feed process) for the purpose of production of L-amino acids. A summary of known culture methods is described in the textbook by Chmiel (Bioprozesstechnik 1. Einführung in die Bioverfahrenstechnik (Gustav Fischer Verlag, Stuttgart, 1991)) or in the textbook by Storhas (Bioreaktoren und periphere Einrichtungen (Vieweg Verlag, Braunschweig/ Wiesbaden, 1994)).

The culture medium to be used must meet the requirements of the particular strains in a suitable manner. Descriptions of culture media for various microorganisms are contained in the handbook "Manual of Methods for General Bacteriology" of the American Society for Bacteriology (Washington D.C., USA, 1981).

Sugars and carbohydrates, such as e.g. glucose, sucrose, lactose, fructose, maltose, molasses, starch and cellulose, oils and fats, such as, for example, soya oil, sunflower oil, groundnut oil and coconut fat, fatty acids, such as, for example, palmitic acid, stearic acid and linoleic acid, alcohols, such as, for example, glycerol and ethanol, and organic acids, such as, for example, acetic acid, can be used as the source of carbon. These substances can be used individually or as a mixture.

Organic nitrogen-containing compounds, such as peptones, yeast extract, meat extract, malt extract, corn steep liquor, soya bean flour and urea, or inorganic compounds, such as ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate and ammonium nitrate, can be used as the source of nitrogen. The sources of nitrogen can be used individually or as a mixture.

Phosphoric acid, potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts can be used as the source of phosphorus. The culture medium must furthermore comprise salts of metals, such as, for example, magnesium sulfate or iron sulfate, which are necessary for growth. Finally, essential growth substances, such as amino acids and vitamins, can be employed in addition to the abovementioned substances. Suitable precursors can moreover be added to the culture medium. The starting substances mentioned can be added to the culture in the form of a single batch, or can be fed in during the culture in a suitable manner.

Basic compounds, such as sodium hydroxide, potassium hydroxide, ammonia or aqueous ammonia, or acid compounds, such as phosphoric acid or sulfuric acid, can be employed in a suitable manner to control the pH of the culture. Antifoams, such as, for example, fatty acid polyglycol esters, can be employed to control the development of foam. Suitable substances having a selective action, such as, for

example, antibiotics, can be added to the medium to maintain the stability of plasmids. To maintain aerobic conditions, oxygen or oxygen-containing gas mixtures, such as, for example, air, are introduced into the culture. The temperature of the culture is usually 20°C to 45°C, and preferably 25°C to 40°C. Culturing is continued until a maximum of the desired product has formed. This target is usually reached within 10 hours to 160 hours.

Methods for the determination of L-amino acids are known from the prior art. The analysis can thus be carried out, for example, as described by Spackman et al. (Analytical Chemistry, 30, (1958), 1190) by anion exchange chromatography with subsequent ninhydrin derivatization, or it can be carried out by reversed phase HPLC, for example as described by Lindroth et al. (Analytical Chemistry (1979) 51: 1167-1174).

The process according to the invention is used for fermentative preparation of amino acids.

The present invention is explained in more detail in the following with the aid of embodiment examples.

The isolation of plasmid DNA from Escherichia coli and all techniques of restriction, Klenow and alkaline phosphatase treatment were carried out by the method of Sambrook et al. (Molecular Cloning. A Laboratory Manual, 1989, Cold Spring Harbour Laboratory Press, Cold Spring Harbor, NY, USA). Methods for transformation of Escherichia coli are also described in this handbook.

The composition of the usual nutrient media, such as LB or TY medium, can also be found in the handbook by Sambrook et al.

Example 1

Preparation of a genomic cosmid gene library from C. glutamicum ATCC 13032

Chromosomal DNA from C. glutamicum ATCC 13032 was isolated as described by Tauch et al. (1995, Plasmid 33:168-179) and partly cleaved with the restriction enzyme Sau3AI (Amersham Pharmacia, Freiburg, Germany, Product Description Sau3AI, Code no. 27-0913-02). The DNA fragments were dephosphorylated with shrimp alkaline phosphatase (Roche Molecular Biochemicals, Mannheim, Germany, Product Description SAP, Code no. 1758250). The DNA of the cosmid vector SuperCos1 (Wahl et al. (1987), Proceedings of the National Academy of Sciences, USA 84:2160-2164), obtained from Stratagene (La Jolla, USA, Product Description SuperCos1 Cosmid Vector Kit, Code no. 251301) was cleaved with the restriction enzyme XbaI (Amersham Pharmacia, Freiburg, Germany, Product Description XbaI, Code no. 27-0948-02) and likewise dephosphorylated with shrimp alkaline phosphatase.

The cosmid DNA was then cleaved with the restriction enzyme BamHI (Amersham Pharmacia, Freiburg, Germany, Product Description BamHI, Code no. 27-0868-04). The cosmid DNA treated in this manner was mixed with the treated ATCC13032 DNA and the batch was treated with T4 DNA ligase (Amersham Pharmacia, Freiburg, Germany, Product Description T4-DNA-Ligase, Code no.27-0870-04). The ligation mixture was then packed in phages with the aid of Gigapack II XL Packing Extract (Stratagene, La Jolla, USA, Product Description Gigapack II XL Packing Extract, Code no. 200217).

For infection of the E. coli strain NM554 (Raleigh et al. 1988, Nucleic Acid Res. 16:1563-1575) the cells were taken up in 10 mM $MgSO_4$ and mixed with an aliquot of the phage suspension. The infection and titering of the cosmid library were carried out as described by Sambrook et al.

(1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor), the cells being plated out on LB agar (Lennox, 1955, Virology, 1:190) + 100 µg/ml ampicillin. After incubation overnight at 37°C, recombinant individual clones were selected.

Example 2

Isolation and sequencing of the deaD gene

The cosmid DNA of an individual colony was isolated with the Qiaprep Spin Miniprep Kit (Product No. 27106, Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions and partly cleaved with the restriction enzyme Sau3AI (Amersham Pharmacia, Freiburg, Germany, Product Description Sau3AI, Product No. 27-0913-02). The DNA fragments were dephosphorylated with shrimp alkaline phosphatase (Roche Molecular Biochemicals, Mannheim, Germany, Product Description SAP, Product No. 1758250). After separation by gel electrophoresis, the cosmid fragments in the size range of 1500 to 2000 bp were isolated with the QiaExII Gel Extraction Kit (Product No. 20021, Qiagen, Hilden, Germany).

The DNA of the sequencing vector pZero-1, obtained from Invitrogen (Groningen, The Netherlands, Product Description Zero Background Cloning Kit, Product No. K2500-01) was cleaved with the restriction enzyme BamHI (Amersham Pharmacia, Freiburg, Germany, Product Description BamHI, Product No. 27-0868-04). The ligation of the cosmid fragments in the sequencing vector pZero-1 was carried out as described by Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor), the DNA mixture being incubated overnight with T4 ligase (Pharmacia Biotech, Freiburg, Germany). This ligation mixture was then electroporated (Tauch et al. 1994, FEMS Microbiol. Letters, 123:343-7) into the E. coli strain DH5αmcr (Grant, 1990, Proceedings of the National Academy of Sciences, U.S.A.,

87:4645-4649). Letters, 123:343-7) and plated out on LB agar (Lennox, 1955, Virology, 1:190) with 50 µg/ml zeocin.

The plasmid preparation of the recombinant clones was carried out with the Biorobot 9600 (Product No. 900200, Qiagen, Hilden, Germany). The sequencing was carried out by the dideoxy chain termination method of Sanger et al. (1977, Proceedings of the National Academies of Sciences, U.S.A., 74:5463-5467) with modifications according to Zimmermann et al. (1990, Nucleic Acids Research, 18:1067). The "RR dRhodamin Terminator Cycle Sequencing Kit" from PE Applied Biosystems (Product No. 403044, Weiterstadt, Germany) was used. The separation by gel electrophoresis and analysis of the sequencing reaction were carried out in a "Rotiphoresis NF Acrylamide/Bisacrylamide" Gel (29:1) (Product No. A124.1, Roth, Karlsruhe, Germany) with the "ABI Prism 377" sequencer from PE Applied Biosystems (Weiterstadt, Germany).

The raw sequence data obtained were then processed using the Staden program package (1986, Nucleic Acids Research, 14:217-231) version 97-0. The individual sequences of the pZero1 derivatives were assembled to a continuous contig. The computer-assisted coding region analysis were prepared with the XNIP program (Staden, 1986, Nucleic Acids Research, 14:217-231). Further analyses were carried out with the "BLAST search program" (Altschul et al., 1997, Nucleic Acids Research, 25:3389-3402) against the non-redundant databank of the "National Center for Biotechnology Information" (NCBI, Bethesda, MD, USA).

The resulting nucleotide sequence is shown in SEQ ID No. 1. Analysis of the nucleotide sequence showed an open reading frame of 1875 bp, which was called the deaD gene. The deaD gene codes for a polypeptide of 624 amino acids.

SEQUENCE PROTOCOL

<110> Degussa-Hüls AG

<120> New nucleotide sequences which code for the deaD gene

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<140>
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<222> (259)..(2130)
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tctgaggacg ccgactctgc agatgcagac aacgcgagca atgtaatcaa tgagaatgag 180

gactcctcgg aaggtgctaa ccagccttca aacgagtcat cctctacgga agccaaatcc 240

ggcttcgatg cactcgga ctg cca gag cgt gta ctt gac gct gtg cgc aag 291
Met Pro Glu Arg Val Leu Asp Ala Val Arg Lys
1 5 10

gtg ggt tac gaa act cct tcc cca att cag gca caa acc atc cca atc 339
Val Gly Tyr Glu Thr Pro Ser Pro Ile Gln Ala Gln Thr Ile Pro Ile
15 20 25

ctc atg gag ggc cag gat gtt gtt ggt cta gca cag acc ggt acc ggt 387
Leu Met Glu Gly Gln Asp Val Val Gly Leu Ala Gln Thr Gly Thr Gly
30 35 40

aag act gca gct ttc gcg ctg cca atc ctt gcc cgt att gac aag tcc 435
Lys Thr Ala Ala Phe Ala Leu Pro Ile Leu Ala Arg Ile Asp Lys Ser
45 50 55

gtg cgc agc cca cag gca ctt gtg ctt gcc cct acc cgt gag cag gca 483
Val Arg Ser Pro Gln Ala Leu Val Leu Ala Pro Thr Arg Glu Gln Ala
60 65 70 75

ctt cag gtt gct gac tcc ttc caa tcc ttc gct gac cac gtc ggt ggc 531
Leu Gln Val Ala Asp Ser Phe Gln Ser Phe Ala Asp His Val Gly Gly
80 85 90

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ctg aac gtt ctg cca atc tat ggt gga cag gct tac ggc att cag ctc   579
Leu Asn Val Leu Pro Ile Tyr Gly Gly Gln Ala Tyr Gly Ile Gln Leu
            95                  100                 105

tct ggc ctg cgt cgt ggc gct cac atc gtc gtg ggt acc cca ggc cga   627
Ser Gly Leu Arg Arg Gly Ala His Ile Val Val Gly Thr Pro Gly Arg
        110                 115                 120

atc atc gat cac ctc gaa aag ggc tcc ctg gat atc tcc gga ctg cgc   675
Ile Ile Asp His Leu Glu Lys Gly Ser Leu Asp Ile Ser Gly Leu Arg
    125                 130                 135

ttc ctc gtg ctc gat gaa gca gac gag atg ctg aac atg ggc ttc cag   723
Phe Leu Val Leu Asp Glu Ala Asp Glu Met Leu Asn Met Gly Phe Gln
140                 145                 150                 155

gaa gat gtc gag cgc atc ctc gag gac acc cca gac gag aag cag gtt   771
Glu Asp Val Glu Arg Ile Leu Glu Asp Thr Pro Asp Glu Lys Gln Val
                160                 165                 170

gca cta ttc tcc gca acg atg cca aac ggc att cgt cgc ctg tcc aag   819
Ala Leu Phe Ser Ala Thr Met Pro Asn Gly Ile Arg Arg Leu Ser Lys
            175                 180                 185

cag tac ctg aac aac cct gct gaa atc acc gtt aag tcc gag acc agg   867
Gln Tyr Leu Asn Asn Pro Ala Glu Ile Thr Val Lys Ser Glu Thr Arg
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act aac acc aac atc acc cag cgc ttc ctc aac gtt gca cac cgc aac   915
Thr Asn Thr Asn Ile Thr Gln Arg Phe Leu Asn Val Ala His Arg Asn
    205                 210                 215

aag atg gat gca ctg acc cgt att ctc gag gtc acc gag ttt gaa gca   963
Lys Met Asp Ala Leu Thr Arg Ile Leu Glu Val Thr Glu Phe Glu Ala
220                 225                 230                 235

atg atc atg ttc gtg cgc acc aag cac gaa act gaa gaa gtt gct gaa   1011
Met Ile Met Phe Val Arg Thr Lys His Glu Thr Glu Glu Val Ala Glu
                240                 245                 250

aag ctc cgt gca cgc gga ttc tcc gca gca gcc atc aac ggc gac att   1059
Lys Leu Arg Ala Arg Gly Phe Ser Ala Ala Ala Ile Asn Gly Asp Ile
            255                 260                 265

gct cag gca cag cgt gag cgc acc gtc gac cag ctg aag gac ggc cgc   1107
Ala Gln Ala Gln Arg Glu Arg Thr Val Asp Gln Leu Lys Asp Gly Arg
        270                 275                 280

ctg gac atc ctc gtt gca acc gac gtt gca gcc cgt ggt ctt gac gtt   1155
Leu Asp Ile Leu Val Ala Thr Asp Val Ala Ala Arg Gly Leu Asp Val
    285                 290                 295

gag cgc atc tcc cac gtg ctt aac ttc gac att cca aac gac acc gag   1203
Glu Arg Ile Ser His Val Leu Asn Phe Asp Ile Pro Asn Asp Thr Glu
300                 305                 310                 315

tcc tac gtt cac cgc atc ggc cgc acc ggc cgt gca gga cgt acc ggc   1251
Ser Tyr Val His Arg Ile Gly Arg Thr Gly Arg Ala Gly Arg Thr Gly
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atc gag cgc gca acc aac gca cca ctg cac gaa atg gaa ctg cca acc   1347
Ile Glu Arg Ala Thr Asn Ala Pro Leu His Glu Met Glu Leu Pro Thr
        350                 355                 360

gtc gat cag gtc aac gac ttc cgc aag gtc aag ttc gct gac tcc atc   1395
Val Asp Gln Val Asn Asp Phe Arg Lys Val Lys Phe Ala Asp Ser Ile
    365                 370                 375

acc aag tcc ctc gag gac aag cag atg gac ctg ttc cgc acc ctg gtc   1443
Thr Lys Ser Leu Glu Asp Lys Gln Met Asp Leu Phe Arg Thr Leu Val
380                 385                 390                 395

aag gaa tac tcc cag gcc aac gac gtt cct cta gag gac atc gca gcg   1491
Lys Glu Tyr Ser Gln Ala Asn Asp Val Pro Leu Glu Asp Ile Ala Ala
                400                 405                 410

gca ctg gca acc cag gca cag tcc ggc gac ttc ctg ctc aag gag ctc   1539
Ala Leu Ala Thr Gln Ala Gln Ser Gly Asp Phe Leu Leu Lys Glu Leu
            415                 420                 425

cca cca gag cgc cgt gag cgc aac gac cgc cgt cgt gac cgt gac ttc   1587
Pro Pro Glu Arg Arg Glu Arg Asn Asp Arg Arg Arg Asp Arg Asp Phe
        430                 435                 440

gac gac cgt ggt gga cgt gga cgc gac cgt gac cgt ggc gac cgc gga   1635
Asp Asp Arg Gly Gly Arg Gly Arg Asp Arg Asp Arg Gly Asp Arg Gly
    445                 450                 455

gat cgt ggc tca cgc ttc gac cgc gac gac gag aac ctg gca acc tac   1683
Asp Arg Gly Ser Arg Phe Asp Arg Asp Asp Glu Asn Leu Ala Thr Tyr
460                 465                 470                 475

cgc ctc gca gtg ggc aag cgc cag cac atc cgc cca ggc gca atc gtt   1731
Arg Leu Ala Val Gly Lys Arg Gln His Ile Arg Pro Gly Ala Ile Val
                480                 485                 490

ggt gca ctt gcc aac gaa ggt ggc ctg aac tcc aag gac ttc ggc cgc   1779
Gly Ala Leu Ala Asn Glu Gly Gly Leu Asn Ser Lys Asp Phe Gly Arg
            495                 500                 505

atc acc atc gca gcc gac cac acc ctg gtt gaa ctg cca aag gat ctc   1827
Ile Thr Ile Ala Ala Asp His Thr Leu Val Glu Leu Pro Lys Asp Leu
        510                 515                 520

cca cag agc gtt ctt gac aac ctg cgc gac acc cgc atc tcc ggc cag   1875
Pro Gln Ser Val Leu Asp Asn Leu Arg Asp Thr Arg Ile Ser Gly Gln
    525                 530                 535

ctc atc aac ata gaa cgc gac tcc ggt gga cgc cca cca cgc cgc ttc   1923
Leu Ile Asn Ile Glu Arg Asp Ser Gly Gly Arg Pro Pro Arg Arg Phe
540                 545                 550                 555
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gag cgc gat gac cgt ggc gga cgc ggc gga ttc cgc ggc gac cgt gat   1971
Glu Arg Asp Asp Arg Gly Gly Arg Gly Gly Phe Arg Gly Asp Arg Asp
                560                 565                 570

gac cgc ggt gga cgt gga cgt gac cgt gac gat cgt gga agc cgt gga   2019
Asp Arg Gly Gly Arg Gly Arg Asp Arg Asp Asp Arg Gly Ser Arg Gly
            575                 580                 585

ggt ttc cgc ggt gga cgt gac cgt gat gat cgt ggc gga cgc ggt gga   2067
Gly Phe Arg Gly Gly Arg Asp Arg Asp Asp Arg Gly Gly Arg Gly Gly
        590                 595                 600

ttc cgc gga cgc gac gac cgc gga gac cgt ggt ggc cgt ggc ggt tac   2115
Phe Arg Gly Arg Asp Asp Arg Gly Asp Arg Gly Gly Arg Gly Gly Tyr
    605                 610                 615

cgt ggc gga cgc gac taagagttcg ttttagcttc agctcaggtt ttcgcctgag   2170
Arg Gly Gly Arg Asp
620

tctggtgctt agctagaaaa atccgttgct ctctctttac tgagagggca acggattttt 2230

tctgttttct taggctttgg ttcttggggg atcttggggg aggaattcta ggaacttaga 2290

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             20                  25                  30

Asp Val Val Gly Leu Ala Gln Thr Gly Thr Gly Lys Thr Ala Ala Phe
         35                  40                  45

Ala Leu Pro Ile Leu Ala Arg Ile Asp Lys Ser Val Arg Ser Pro Gln
     50                  55                  60

Ala Leu Val Leu Ala Pro Thr Arg Glu Gln Ala Leu Gln Val Ala Asp
 65                  70                  75                  80

Ser Phe Gln Ser Phe Ala Asp His Val Gly Gly Leu Asn Val Leu Pro
                 85                  90                  95

Ile Tyr Gly Gly Gln Ala Tyr Gly Ile Gln Leu Ser Gly Leu Arg Arg
            100                 105                 110

Gly Ala His Ile Val Val Gly Thr Pro Gly Arg Ile Ile Asp His Leu
        115                 120                 125
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Glu Lys Gly Ser Leu Asp Ile Ser Gly Leu Arg Phe Leu Val Leu Asp
    130                 135                 140

Glu Ala Asp Glu Met Leu Asn Met Gly Phe Gln Glu Asp Val Glu Arg
145                 150                 155                 160

Ile Leu Glu Asp Thr Pro Asp Glu Lys Gln Val Ala Leu Phe Ser Ala
                165                 170                 175

Thr Met Pro Asn Gly Ile Arg Arg Leu Ser Lys Gln Tyr Leu Asn Asn
            180                 185                 190

Pro Ala Glu Ile Thr Val Lys Ser Glu Thr Arg Thr Asn Thr Asn Ile
        195                 200                 205

Thr Gln Arg Phe Leu Asn Val Ala His Arg Asn Lys Met Asp Ala Leu
    210                 215                 220

Thr Arg Ile Leu Glu Val Thr Glu Phe Glu Ala Met Ile Met Phe Val
225                 230                 235                 240

Arg Thr Lys His Glu Thr Glu Glu Val Ala Glu Lys Leu Arg Ala Arg
                245                 250                 255

Gly Phe Ser Ala Ala Ala Ile Asn Gly Asp Ile Ala Gln Ala Gln Arg
            260                 265                 270

Glu Arg Thr Val Asp Gln Leu Lys Asp Gly Arg Leu Asp Ile Leu Val
        275                 280                 285

Ala Thr Asp Val Ala Ala Arg Gly Leu Asp Val Glu Arg Ile Ser His
    290                 295                 300

Val Leu Asn Phe Asp Ile Pro Asn Asp Thr Glu Ser Tyr Val His Arg
305                 310                 315                 320

Ile Gly Arg Thr Gly Arg Ala Gly Arg Thr Gly Glu Ala Ile Leu Phe
                325                 330                 335

Val Thr Pro Arg Glu Arg Arg Met Leu Arg Ser Ile Glu Arg Ala Thr
            340                 345                 350

Asn Ala Pro Leu His Glu Met Glu Leu Pro Thr Val Asp Gln Val Asn
        355                 360                 365

Asp Phe Arg Lys Val Lys Phe Ala Asp Ser Ile Thr Lys Ser Leu Glu
    370                 375                 380

Asp Lys Gln Met Asp Leu Phe Arg Thr Leu Val Lys Glu Tyr Ser Gln
385                 390                 395                 400

Ala Asn Asp Val Pro Leu Glu Asp Ile Ala Ala Ala Leu Ala Thr Gln
                405                 410                 415

Ala Gln Ser Gly Asp Phe Leu Leu Lys Glu Leu Pro Pro Glu Arg Arg
            420                 425                 430

Glu Arg Asn Asp Arg Arg Arg Asp Arg Asp Phe Asp Asp Arg Gly Gly
        435                 440                 445
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Arg Gly Arg Asp Arg Asp Arg Gly Asp Arg Gly Asp Arg Gly Ser Arg
    450                 455                 460

Phe Asp Arg Asp Asp Glu Asn Leu Ala Thr Tyr Arg Leu Ala Val Gly
465                 470                 475                 480

Lys Arg Gln His Ile Arg Pro Gly Ala Ile Val Gly Ala Leu Ala Asn
                485                 490                 495

Glu Gly Gly Leu Asn Ser Lys Asp Phe Gly Arg Ile Thr Ile Ala Ala
            500                 505                 510

Asp His Thr Leu Val Glu Leu Pro Lys Asp Leu Pro Gln Ser Val Leu
        515                 520                 525

Asp Asn Leu Arg Asp Thr Arg Ile Ser Gly Gln Leu Ile Asn Ile Glu
    530                 535                 540

Arg Asp Ser Gly Gly Arg Pro Pro Arg Arg Phe Glu Arg Asp Asp Arg
545                 550                 555                 560

Gly Gly Arg Gly Gly Phe Arg Gly Asp Arg Asp Asp Arg Gly Gly Arg
                565                 570                 575

Gly Arg Asp Arg Asp Asp Arg Gly Ser Arg Gly Gly Phe Arg Gly Gly
            580                 585                 590

Arg Asp Arg Asp Asp Arg Gly Gly Arg Gly Gly Phe Arg Gly Arg Asp
        595                 600                 605

Asp Arg Gly Asp Arg Gly Gly Arg Gly Gly Tyr Arg Gly Gly Arg Asp
    610                 615                 620
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**Patent claims**

1. An isolated polynucleotide from coryneform bacteria, comprising a polynucleotide sequence which codes for the deaD gene, chosen from the group consisting of

   a) polynucleotide which is identical to the extent of at least 70% to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2,

   b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70% to the amino acid sequence of SEQ ID No. 2,

   c) polynucleotide which is complementary to the polynucleotides of a) or b), and

   d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b) or c),

   the polypeptide preferably having the activity of DNA/RNA helicase.

2. The polynucleotide as claimed in claim 1, wherein the polynucleotide is a preferably recombinant DNA which is capable of replication in coryneform bacteria.

3. The polynucleotide as claimed in claim 1, wherein the polynucleotide is an RNA.

4. The polynucleotide as claimed in claim 2, comprising the nucleic acid sequence as shown in SEQ ID No. 1.

5. The DNA as claimed in claim 2 which is capable of replication, comprising

(i) the nucleotide sequence shown in SEQ ID No. 1, or

(ii) at least one sequence which corresponds to sequence (i) within the range of the degeneration of the genetic code, or

(iii) at least one sequence which hybridizes with the sequence complementary to sequence (i) or (ii), and optionally

(iv) sense mutations of neutral function in (i).

6. The DNA as claimed in claim 5 which is capable of replication, wherein the hybridization is carried out under a stringency corresponding to at most 2x SSC.

7. The polynucleotide sequence as claimed in claim 1, which codes for a polypeptide which comprises the amino acid sequence shown in SEQ ID No. 2.

8. Coryneform bacteria in which the deaD gene is attenuated, in particular eliminated.

9. A process for the fermentative preparation of L-amino acids, in particular L-lysine, wherein the following steps are carried out:

a) fermentation of the coryneform bacteria which produce the desired L-amino acid and in which at least the deaD gene or nucleotide sequences which code for it are attenuated, in particular eliminated;

b) concentration of the L-amino acid in the medium or in the cells of the bacteria, and

c) isolation of the L-amino acid.

10. The process as claimed in claim 9, wherein bacteria in which further genes of the biosynthesis pathway of the desired L-amino acid are additionally enhanced are employed.

11. The process as claimed in claim 9, wherein bacteria in which the metabolic pathways which reduce the formation of the desired L-amino acid are at least partly eliminated are employed.

12. The process as claimed in claim 9, wherein the expression of the polynucleotide(s) which code(s) for the deaD gene is attenuated, in particular eliminated.

13. The process as claimed in claim 9, wherein the catalytic properties of the polypeptide (enzyme protein) for which the polynucleotide deaD codes are reduced.

14. The process as claimed in claim 9, wherein for the preparation of L-amino acids, coryneform microorganisms in which at the same time one or more of the genes chosen from the group consisting of

14.1 the dapA gene which codes for dihydrodipicolinate synthase,

14.2 the gap gene which codes for glyceraldehyde 3-phosphate dehydrogenase,

14.3 the tpi gene which codes for triose phosphate isomerase,

14.4 the pgk gene which codes for 3-phosphoglycerate kinase,

14.5 the zwf gene which codes for glucose 6-phosphate dehydrogenase,

14.6 the pyc gene which codes for pyruvate carboxylase,

14.7 the mqo gene which codes for malate-quinone oxidoreductase,

14.8 the lysC gene which codes for a feed-back resistant aspartate kinase,

14.9 the lysE gene which codes for lysine export,

14.10 the hom gene which codes for homoserine dehydrogenase

14.11 the ilvA gene which codes for threonine dehydratase or the ilvA(Fbr) allele which codes for a feed back resistant threonine dehydratase,

14.12 the ilvBN gene which codes for acetohydroxy-acid synthase,

14.13 the ilvD gene which codes for dihydroxy-acid dehydratase,

14.14 the zwa1 gene which codes for the Zwa1 protein

is or are enhanced or over-expressed are fermented.

15. The process as claimed in claim 9, wherein for the preparation of L-amino acids, coryneform microorganisms in which at the same time one or more of the genes chosen from the group consisting of

15.1 the pck gene which codes for phosphoenol pyruvate carboxykinase,

15.2 the pgi gene which codes for glucose 6-phosphate isomerase,

15.3 the poxB gene which codes for pyruvate oxidase

15.4 the zwa2 gene which codes for the Zwa2 protein

is or are attenuated are fermented.

16. Coryneform bacteria which contain a vector which carries parts of the polynucleotide as claimed in claim 1, but at least 15 successive nucleotides of the sequence claimed.

17. The process as claimed in one or more of the preceding claims, wherein microorganisms of the species Corynebacterium glutamicum are employed.

18. A process for discovering RNA, cDNA and DNA in order to isolate nucleic acids or polynucleotides or genes which code for DNA/RNA helicase or have a high similarity with the sequence of the deaD gene, wherein the polynucleotide comprising the polynucleotide sequences as claimed in claims 1, 2, 3 or 4 is employed as hybridization probes.

**Abstract**

The invention relates to an isolated polynucleotide comprising a polynucleotide sequence chosen from the group consisting of

a) polynucleotide which is identical to the extent of at least 70% to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2,

b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70% to the amino acid sequence of SEQ ID No. 2,

c) polynucleotide which is complementary to the polynucleotides of a) or b), and

d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b) or c),

and a process for the fermentative preparation of L-amino acids using coryneform bacteria in which at least the deaD gene is present in attenuated form, and the use of polynucleotides which comprise the sequences according to the invention as hybridization probes.